

Development and Evaluation of an Enzyme-Linked Immunosorbent Assay for Serum Vi Antibodies for Detection of Chronic *Salmonella typhi* Carriers

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An enzyme-linked immunosorbent assay (ELISA) measuring, in serum, immunoglobulin G (IgG), IgM, and IgA to Vi capsular polysaccharide antigen that was tyraminated (Vi-Tyr) to increase its binding efficiency to microtiter plates was compared with the standard passive hemagglutination assay (PHA) as a screening test for chronic *Salmonella typhi* carriers. Initially, three populations were evaluated: 22 healthy U.S. adults, 17 young Chilean adults with acute typhoid fever, and 51 Chileans who had bacteriologically confirmed *S. typhi* chronic carriage. IgG-specific Vi-Tyr antibodies were preferentially present in the *S. typhi* chronic carrier state. A total of 44 of 51 (86%) chronic carriers, 0 of 22 (0%) healthy U.S. adults, and 2 of 17 (12%) Chileans with acute typhoid fever had reciprocal IgG Vi-Tyr ELISA antibody titers in serum of ≥ 200 . The IgG Vi-Tyr ELISA was then compared with the PHA as a screening test for chronic carriers in 141 Chilean female food handlers. One woman was serologically incriminated as a carrier by both the IgG ELISA and PHA; her coprocultures were positive for *S. typhi*. One other woman, identified as a carrier by PHA, was negative by culture and IgG ELISA. The IgG Vi-Tyr ELISA is as sensitive as the PHA (86 versus 76%) and as specific (95 versus 95%) in screening for chronic carriers.

Asymptomatic excretion of *Salmonella typhi* in stools for greater than 1 year following an episode of acute typhoid fever occurs in approximately 3% of adults (9). These asymptomatic chronic biliary carriers represent an important reservoir of *S. typhi* and have been responsible for outbreaks of acute typhoid fever (13). Detection of carriers, therefore, is an important aspect of typhoid fever control. Bacteriological confirmation of the chronic carrier state requires either multiple stool cultures or cultures of bile or bile-stained duodenal fluid. These procedures are not amenable to large-scale screening (6, 7, 10). In addition, because chronic biliary carriers are often intermittent or light fecal *S. typhi* excretors, multiple bacteriological examinations are usually required to make a reliable diagnosis (3, 6, 10). For these reasons, serological screening for the carrier state of *S. typhi* in areas of typhoid endemicity is preferable.

The passive hemagglutination assay (PHA) with Vi antigen from *Citrobacter freundii* or *S. typhi* has been found to be both sensitive and specific for the screening of the chronic carrier state of *S. typhi* in endemic and nonendemic areas (8, 11). However, for this assay it is required that test sera be preabsorbed with sheep erythrocytes, which is inconvenient for screening large populations. Attempts at using an enzyme-linked immunosorbent assay (ELISA) for the detection of the carrier state have been hampered by the poor binding of the Vi antigen to microtiter plates. Some researchers, using immune sera as the capture reagent for the Vi antigen, have had some success in detecting specific immunoglobulin G (IgG) (2, 5). However, large amounts of a standard immune serum are needed and may not be readily available.

Highly purified Vi polysaccharide from *C. freundii* was tyraminated (Vi-Tyr) in an attempt to enhance binding of the polysaccharide to plastic microtiter plates (12). This Vi-Tyr was then used in the development of an ELISA which was compared with the PHA as a screening test for typhoid carriers in a typhoid-endemic area. The ELISA was adapted to assess the relative occurrence of Vi-specific IgG, IgM, and IgA in the carrier state.

MATERIALS AND METHODS

Subjects. To standardize the Vi-Tyr ELISA, three groups of subjects were examined: 22 healthy young adults from the United States, 17 young Chilean adults admitted to the Infectious Diseases Hospital in Santiago with bacteriologically confirmed acute typhoid fever, and 51 asymptomatic *S. typhi* carriers from Chile. These chronic carriers had bacteriologically confirmed typhoid fever 1 to 4 years previously, and at the time of the study, *S. typhi* was bacteriologically isolated from them.

Once a serum dilution for screening carriers was determined with these known populations, 141 Chilean female food handlers (age, 25 to 65 years) were screened blindly for *S. typhi* chronic carriage by the Vi-Tyr ELISA and PHA. *S. typhi* carriage in this group was confirmed by coprocultures.

Specimens. One serum sample was obtained from each subject in all groups, except for the subjects with acute typhoid fever, from whom serum was obtained on admission to the hospital and 21 days later. Those subjects with acute typhoid fever and those with chronic *S. typhi* carriage underwent a bacteriological evaluation consisting of three stool cultures obtained on consecutive days and one duodenal fluid culture obtained by a gelatin-encapsulated string device (1). Two coprocultures were obtained on successive

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days from the healthy female food handlers to confirm *S. typhi* carriage. All samples were inoculated onto MacConkey, Wilson-Blair, and salmonella-shigella agar (Difco Laboratories, Detroit, Mich.) and into Selenite-F Enrichment broth (BBL Microbiology Systems, Cockeysville, Md.). *S. typhi* was recovered and identified by standard biochemical and serological reactions (4).

Vi-Tyr antigen. Vi-Tyr polysaccharide has been described previously (12). Briefly, tyramine (30 mg/ml) was added to 10 mg of Vi in the presence of carbodiimide and incubated at pH 4.9 to 5.1 for 3 h. The resultant reaction mixture was dialyzed and purified by gel exclusion through a G-100 Sephadex column (Pharmacia Fine Chemicals, Piscataway, N.J.).

Standardization of the Vi-Tyr ELISA. Serum samples from 16 known chronic typhoid carriers and from 6 healthy U.S. volunteers were used as the positive and negative reference sera, respectively, to establish a standard curve for each isotype-specific Vi-Tyr ELISA. These samples were assayed 12 different times at twofold dilutions, starting at 1:25 and ending at 1:3,200, by the following method. The wells of Immulon I (Dynatech Laboratories, Inc., Alexandria, Va.) plates were incubated at 4°C overnight with 0.1-ml fractions of Vi-Tyr antigen in phosphate-buffered saline (PBS; pH 7.3). The wells were washed 5 times with PBS containing 0.05% Tween 20 (PBS-Tween) and then incubated at 37°C for 1 h with 0.1 ml of human serum diluted in PBS-Tween containing 1% nonimmune goat serum and 1% fetal bovine serum. The wells were then washed 5 times with PBS-Tween and incubated for 1 h at 37°C with heavy-chain-specific antibody to human IgG, IgM, and IgA conjugated to alkaline phosphatase (Kirkegaard and Perry, Gaithersburg, Md.) diluted in PBS-Tween. After the wells were washed, they were incubated at room temperature with 0.1 ml of *p*-nitrophenyl phosphate (1 mg/ml) in 10% diethanolamine buffer (pH 9.8). The A_{405} was monitored.

Saturation kinetics with several serum samples with high titers, determined by PHA, were determined by using Vi-Tyr coating concentrations of 0.5, 1.0, and 2.0 µg/ml. The specificity of the goat antibody conjugates were examined with purified IgG, IgM fractions in serum, and IgA obtained from milk by filtration through a DEAE Biogel-A (Pharmacia) column.

The PHA was performed for each subject in each group by previously described methods (6). A titer of ≥ 160 was considered to be indicative of the *S. typhi* carrier state.

RESULTS

Assay standardization. The absorbance of the pooled positive serum was determined as a function of the Tyr-Vi antigen coating concentration. Saturation kinetics were observed, and a coating concentration of 1 µg/ml was chosen. The pooled IgG fraction contained 1,450 mg of IgG per dl and less than 1 mg of IgM per dl. The pooled IgM fraction contained 140 mg of IgM per dl and less than 1 mg of IgG per dl. The IgA sample contained 35.5 mg of IgA per dl, and there was less than 1 mg of IgG and IgM per ml. The conjugates were shown to be isotype specific.

A standard curve for IgG-specific Vi with positive sera was linear for absorbance values ranging from 1.2 to 0.1 by using serum dilutions ranging from 1:50 to 1:800 (Fig. 1). The negative serum pool gave optical densities below 0.1 for dilutions as low as 1:25. A dilution of 1:50 of the negative serum pool produced a mean optical density of 0.04 with a standard deviation of 0.01. The cutoff absorbance value

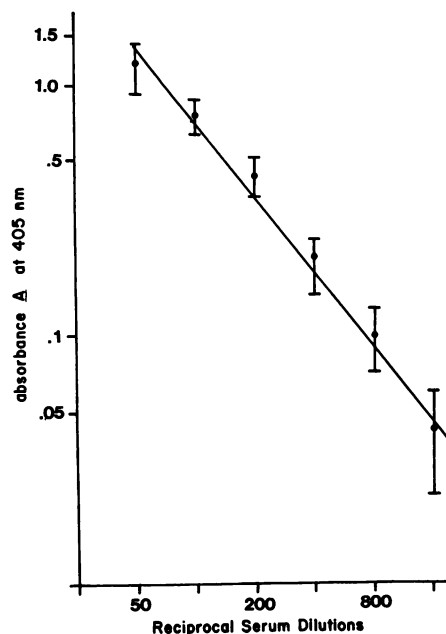


FIG. 1. IgG-specific response to Vi-Tyr antigen in pooled sera from 16 asymptomatic *S. typhi* carriers as measured by ELISA. Antibody results are represented as the mean (●) and 2 standard deviations (bars) compiled from 12 separate runs.

signifying significant IgG-specific Vi was set at an optical density reading of 0.2 since this value is on the linear portion of the curve and well above the background value.

The IgM standard curve for Vi antibody was linear for absorbance values ranging from 1.4 to 0.15, with serum dilutions ranging from 1:25 to 1:400 (data not shown). Because of high background values in the negative serum pool for dilutions of less than 1:100, starting dilutions of 1:100 were used in all samples. A cutoff value of 0.3 was used to determine a positive antibody titer since the absorbance of the negative serum pool, at a 1:100 dilution, was 0.07 ± 0.05 . Similarly, an IgA standard curve was determined as being linear for absorbance values ranging from 0.9 to 0.14, with serum dilutions ranging from 1:50 to 1:400 (data not shown). An absorbance value of 0.15 was conservatively chosen as the cutoff value for a positive antibody titer since the pooled negative sera at a 1:50 dilution gave an optical density of 0.02 ± 0.01 .

Evaluation of the Vi-Tyr ELISA to detect *S. typhi* carriers.

The results of the IgG Vi-Tyr-specific antibody titers in individuals with acute typhoid fever and *S. typhi* carriage and a healthy population are shown in Table 1. Of the 51 chronic carriers tested, 44 (86%) had an IgG Vi-Tyr ELISA titer greater than or equal to 1:200. In contrast, only 12% of the patients with acute typhoid fever and none of the healthy U.S. volunteers had similar titers. An IgG titer of $\geq 1:200$ significantly discriminated among chronic carriers, patients with acute typhoid fever, and a normal U.S. population ($P < 0.00000001$). An IgM-specific Vi titer of >100 was detected in 19 (37%) chronic carriers and in 3 (18%) patients with acute typhoid fever (Table 2). The IgM Vi-Tyr ELISA was unable to discriminate between patients with acute typhoid fever and chronic carriers ($P = 0.2$) and did not increase the detection sensitivity of carriers of the IgG Vi-Tyr ELISA. Although Vi-Tyr-specific IgA was present in 37 (72%) of the chronic carriers, IgA was also detected in patients with acute typhoid fever ($P = 0.2$) (Table 3). ELISA Vi-Tyr antibodies

TABLE 1. Prevalence of IgG-specific Vi

Group description (no. of subjects)	GMT ^a	No. of subjects with an ELISA titer of ^b :			No. of subjects with a PHA titer of ^c :		
		<50	50-100	>200	<40	80	>160
U.S. volunteers (22)	26	21	1	0	21	1	0
Patients with acute typhoid fever (17)	39/44 ^d	15	0	2	14	1	2
Chronic carriers (51)	468	6	1	44	7	5	39
Food handlers (141)	NA ^e	140	0	1 ^f	139	0	2

^a Reciprocal geometric mean Vi titer.^b Number of subjects with a given reciprocal Vi titer.^c Number of subjects with a given reciprocal PHA titer.^d Titer on admission/titer on follow-up.^e NA, Not applicable.^f The one subject identified bacteriologically as being a chronic carrier.

of all three immunoglobulin classes were seen with equal but low frequency in the admission and follow-up serum samples obtained from patients with acute typhoid fever.

To assess the applicability of the IgG Vi-Tyr ELISA in a typhoid-endemic area, 141 Chilean female food handlers were screened by ELISA, PHA, and two coprocultures. Of these 141 women tested, 1 had an IgG ELISA titer of ≥ 200 and 2 women, one of whom also had a positive ELISA titer, had a PHA titer of ≥ 160 (Table 1). Of these two women serologically identified as possible carriers, only the one woman who was positive by the Vi-Tyr IgG ELISA was confirmed to be a carrier by culture.

The sensitivity of the IgG Vi ELISA titer of ≥ 200 in screening for chronic *S. typhi* carriage, as determined by analyzing the results obtained with the 51 known chronic carriers, was 86% compared with 76% by PHA, using a titer of ≥ 160 . The specificity of the IgG-specific Vi-Tyr ELISA in screening for chronic carriers when healthy U.S. volunteers and patients with acute typhoid fever were used was 95%, which was equal to that obtained by PHA.

DISCUSSION

Since humans are the only reservoir of *S. typhi*, the detection of carriers is necessary for the control of typhoid fever. In areas of typhoid endemicity, screening for chronic typhoid carriers by serological means is of practical importance since bacteriological screening is expensive and logistically difficult to perform. Vi-Tyr provides sufficient binding of the antigen for detection of Vi-specific antibodies by ELISA. Although our ELISA and PHA were comparable in terms of sensitivity and specificity, we found the IgG-specific Vi-Tyr ELISA to be superior to our previously reported PHA for the detection of *S. typhi* carriers in terms of rapidity and ease of performance (1, 12). The ELISA

TABLE 2. Prevalence of IgM-specific Vi

Group description (no. of subjects)	GMT ^a	No. of subjects with an ELISA titer of ^b :	
		<100	>100
U.S. volunteers (22)	43	21	1
Patients with acute typhoid fever (17)	34/40 ^c	14	3
Chronic carriers (51)	64	32	19

^a Reciprocal geometric mean Vi titer.^b Number of subjects with a given reciprocal titer.^c Titer on admission/titer on follow-up.

TABLE 3. Prevalence of IgA-specific Vi

Group description (no. of subjects)	GMT ^a	No. of subjects with an ELISA titer of ^b :	
		<50	>50
U.S. volunteers (22)	18	20	2
Patients with acute typhoid fever (17)	50/51 ^c	6	11
Chronic carriers (51)	52	14	37

^a Reciprocal geometric mean titer.^b Number of subjects with a given reciprocal titer.^c Titer on admission/titer on follow-up.

described in this report is preferable to previously reported IgG ELISAs because tyramination allows for direct binding of the Vi antigen to the solid phase. In other assays in which immune sera are used as the specific capture for the Vi antigen the running time of the assay is increased, the generation of standard immune sera to be used as capture antibody is mandated, and ultimately, the cost of the assay is increased (2, 5).

Analysis of the different classes of antibody involved in the chronic carrier state has shown that Vi antibody of the IgG class is present most frequently. IgM and IgA Vi antibodies, although seen in chronic carriers, cannot be used to differentiate persons with acute or chronic *S. typhi* infection. It is probable that the IgG response to Vi present in carriers reflects prolonged immunological stimulation. It is interesting that a IgA Vi response is present in serum in both the acute and chronic forms of *S. typhi* infection. This seems reasonable since *S. typhi* participates in an enterohepatic circuit in the pathogenesis of acute typhoid fever and also is a primary occupant of the biliary system in chronic infection. Further work on subclass specificities in both the IgG and IgA responses to Vi antigen in the acute and chronic forms of *S. typhi* infections may help elucidate other possible immunological differences in these two disease states.

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